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# REPRODUCIBLE SAMPLE PREPARATION METHOD FOR QUANTITATIVE STAIN DETECTION

# CROSS REFERENCE TO RELATED APPLICATION

This Application claims filing benefit of U.S. Provisional Patent Application Ser. No. 62/152,075 having a filing date of Apr. 24, 2015, which is incorporated herein by reference <sup>10</sup> for all purposes

## GOVERNMENT SUPPORT CLAUSE

This invention was made with government support under 15 2011-IJ-CX-K055 awarded by National Institute of Justice. The government has certain rights in the invention.

#### **BACKGROUND**

Blood stains, which are among the traces encountered most frequently at crime scenes, are important for potential extraction and amplification of DNA for suspect identification, as well for spatter pattern analysis to reveal a sequence of events. Estimating the age of blood stains with good 25 accuracy and precision has been an elusive goal for forensic investigations. Estimates of blood stain age can contribute to verify witness' statements, limit the number of suspects and confirm alibis.

Blood is composed of plasma (~53%), platelets (<1%), 30 white blood cells (~1%), and red blood cells (~45%). Hemoglobin, an oxygen carrying protein, makes up about 90% of dried blood content. In healthy blood, hemoglobin exists in two forms: deoxyhemoglobin (Hb), which is without oxygen, and oxyhemoglobin (HbO $_2$ ), which is saturated 35 with oxygen. When blood is exposed to air, Hb is completely saturated with oxygen and converts to HbO $_2$ . HbO $_2$  will irreversibly oxidize to methemoglobin (met-Hb). After that, met-Hb will denature to hemichrome (HC). During these process, changes in the secondary structure of the protein will take place. Hemoglobin is about 80% α-helix type proteins, while the other 20% are unordered coils. After aging, hemoglobin contains 60% α-helix type proteins, 30% β-sheet type proteins and 10% other types.

Many stain detection techniques exist (luminol, Bluestar®, fluorescein, hemascein, etc.). However, their limits of detection are not agreed upon and they are unable to be quantitatively compared to one another due to the inability to reproducibly create stain samples. Fourier Transform Infrared (FT-IR) spectrometry was developed to overcome 50 the limitations encounter with the slow scanning of dispersive instruments. FT-IR employed an interferometer to produce a interferogram, which allows all of the infrared frequencies been detected simultaneously. The signal can be measured on the order of one second or so. The measured 55 signal is digitized and then transformed from the time domain to the frequency domain. The infrared spectrum is then presented as a plot of absorbance vs. frequency.

However, one main issue still exists. The stain samples are currently made without regard to the effects of different 60 stain dilutions and substrate properties. Thus, stain detection limits are imprecisely assigned to stain detection techniques, making it difficult to compare stain detection techniques to one another.

Further, many recent studies have attempted to assign 65 limits of detection and/or compare the ability of different stain detection techniques. For studies like these to be

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successful, a method needs to exist which allows reproducible creation of stain samples. Currently, dilutions of stains are made and applied in constant aliquots, but no consideration is given to the effect diluting a liquid has on its behavior when applied to fabric. Generally, the more dilute a liquid, the further the liquid spreads when applied to a substrate. Additionally, consideration has not been given to the affect different substrates have on the spread of applied liquids. For example, a liquid of the same dilution and volume will spread to a smaller area on densely packed cotton than on a loosely woven silk. Both aforementioned phenomena affect the true dilution of the stain. The absence of a technique which controls the liquid-fabric interaction and allows production of reproducible stains has made experiments of this nature hugely imprecise. Consequently, vast ranges of detection limits have been assigned to various stain detection techniques. For example, luminol has been reported to have a bloodstain detection limit of five-millions times dilute (5) to one-hundred times dilute (4).

## BRIEF DESCRIPTION OF THE DRAWINGS

A full and enabling disclosure of the present invention, including the best mode thereof to one skilled in the art, is set forth more particularly in the remainder of the specification, which includes reference to the accompanying figures, in which:

FIG. 1 is a cross-sectional view of an exemplary fabric after printing an inert polymeric composition to form the inert polymeric coating; and

FIG. 2 is a top-down view of the exemplary fabric of FIG.

FIG. 3 illustrates an exemplary method of formation of a stain barrier utilizing a 3D printer.

Repeat use of reference characters in the present specification and drawings is intended to represent the same or analogous features or elements of the present invention.

# DETAILED DESCRIPTION

Reference now will be made to the embodiments of the invention, one or more examples of which are set forth below. Each example is provided by way of an explanation of the invention, not as a limitation of the invention. In fact, it will be apparent to those skilled in the art that various modifications and variations can be made in the invention without departing from the scope or spirit of the invention. For instance, features illustrated or described as one embodiment can be used on another embodiment to yield still a further embodiment. Thus, it is intended that the present invention cover such modifications and variations as come within the scope of the appended claims and their equivalents. It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only, and is not intended as limiting the broader aspects of the present invention, which broader aspects are embodied exemplary constructions.

A stain-barrier is generally provided, along with methods of its application to a fabric. The stain barrier is easily applied to fabric samples via 3-D printing methods, and limits the amount of fabric with which deposited liquid is able to interact. This stain barrier greatly reduces unwanted variability between samples of different dilution or fabric type so that limits of stain detection can be assigned more accurately and precisely and stain detection techniques can be transparently compared. Thus, the effect of stain-dilution and substrate is minimized by application of the stain-barrier